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Applicants: FLACK et al

Serial No.: 07/551,353 Group: 125

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For: GOSSYPOL FOR THE TREATMENT OF CANCER

DECLARATION UNDER 37 C.F.R. 1.132

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

February 12, 1992

Sir:

I, Mary R. Flack, residing at 12914 Ruxton Road, Silver Spring, Maryland, 20904, hereby declare and state as follows:

1. I graduated from Wayne State University School of Medicine, Detroit, Michigan and received the MD Degree in 1984. My professional credentials are summarized in the Curriculum Vitae attached hereto.

2. I am presently employed as a Senior Clinical Investigator, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.

3. I am one of the inventors of the invention disclosed in the present U.S. Patent Application relating to the use of gossypol for the treatment of cancer. I am therefore fully aware of what the present invention encompasses.

4. I have reviewed the outstanding Office Action mailed on September 12, 1991 in connection with application Serial No. 07/551,353. In view of this Office Action in the above-identified application, I wish to present the two documents attached hereto

containing further experimental evidence demonstrating the effectiveness of gossypol acetic acid in the treatment of human cancer.

The attached document entitled "Gossypol in the Treatment of Metastatic Adrenal Cancer" is being submitted to the New England Journal of Medicine, and describes the treatment of 21 patients with metastatic adrenal cancer using oral gossypol acetic acid. While the term gossypol is used therein, it is used as an abbreviation for gossypol acetic acid as indicated at page 2 of this document, in the section entitled "Gossypol Administration". Three of the patients treated did not receive an adequate trial with gossypol acetic acid due to the terminal nature of their illness. Of the 18 remaining patients who received an adequate trial, three exhibited partial tumor responses, one exhibited a minor tumor response, and one exhibited stabilization of disease. These responses occurred in patients who had previously failed to respond to treatment with other drugs. These patients exhibited significant clinical improvement that has enhanced the quality of their life, and perhaps increased their survival. The response rate disclosed therein is similar to that obtained with other agents available for adrenal cancer, while the side effects of gossypol acetic acid were much less than those reported for these other drugs.

The second document attached hereto as part of the present Declaration is a chapter from the textbook entitled Cancer Medicine

authored by myself and George P. Chrousos. This chapter emphasizes that the prognosis for patients with adrenal cancer is extremely poor. The five-year survival rate for patients with metastatic disease is less than 20%. The drugs currently available for treating adrenal cancer provide a response rate of only 10-20%, and cause significant side effects. Thus, in comparison to the current state of therapy, a drug such as gossypol acetic acid, that brings about tumor regression and clinical improvement without significant toxicity, is clearly an improvement in the treatment of adrenal cancer.

It is declared by the undersigned that all statements made herein of the undersigned's own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that the statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such willful, false statements may jeopardize the validity of this application or any patent issuing thereon.

Mary R. Flack

Mary R. Flack, M.D.

Dated this 1 day of April, 1992.

Gossypol in the Treatment of Metastatic Adrenal Cancer

Running Head: Gossypol in Adrenal Cancer

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Abstract

Medical treatment of metastatic adrenal cancer has been largely unsuccessful and associated with considerable toxicity. We have previously shown that gossypol, a plant toxin, inhibits the growth of human adrenal cancer cells in nude mice. We therefore examined the efficacy and toxicity of oral gossypol as a treatment for adrenal cancer in humans. Twenty-one patients with metastatic adrenal cancer received oral gossypol at doses of 30-70 mg/d. Three patients died of their disease without receiving sufficient gossypol to achieve detectable drug levels, and were thus eliminated from the final analysis. Of the eighteen patients who completed at least six weeks of treatment, three patients had partial tumor responses, lasting from several months to over one year, one patient had a minor response followed by resection of her remaining disease, one patient had stable disease, and thirteen patients had disease progression. All patients who responded to gossypol had previously failed to respond to other chemotherapeutic agents. The side effects of gossypol were generally minor and well tolerated, with the only serious side effect being transient abdominal ileus that responded to withholding the drug and restarting it at a lower dose. We conclude that gossypol can be used relatively safely at doses of 0.6-0.8 mg/kg/d on an outpatient basis for the treatment of metastatic adrenal cancer. The response rate is similar to the other agents currently available for adrenal cancer and responses were seen in patients who had previously failed other chemotherapeutic regimens. This study provides the first evidence that gossypol has *in vivo* activity against cancer in humans, indicating the need for further investigation of gossypol as an anti-tumor agent.

Introduction

Adrenal cancer is a rare, fatal malignancy for which medical therapy has been largely unsuccessful. Ortho-para'DDT (mitotane) and other chemotherapeutic regimens have a partial response rate of only 10-20%, have serious side effects, and do not prolong survival (1, 2). Thus, new medical therapies for metastatic adrenal cancer are needed. We have previously shown that gossypol, a spermatotoxin derived from crude cottonseed oil, inhibits the growth of human adrenocortical tumors *in vivo* in nude mice (3). Previous animal studies suggest that intraperitoneal gossypol has anti-tumor activity against ehrlich ascites tumor and mouse mammary carcinoma, but the toxicity of gossypol in these studies was considerable (4, 5). When we used oral gossypol, however, in our nude mice studies, we did not see any adverse effects of gossypol itself. In fact, the survival of the gossypol treated animals was improved over non-treated controls. In large studies of normal volunteers in China receiving oral gossypol for contraception, relatively few side effects were reported (6, 7). Thus, we examined the efficacy and toxicity of oral gossypol as a treatment for metastatic adrenal cancer in humans.

Methods

Patient Selection

Twenty-one patients with metastatic adrenal cancer were enrolled in the study. Fourteen patients were evaluated at the clinical center of the National Institutes of Health (NIH) between Sept., 1989 and Jan, 1991 and seven patients were evaluated at the New York Hospital-Cornell Medical Center (NYH-CMC) between Feb, 1990 and May, 1991. The initial evaluation included a complete history and physical exam, routine blood studies for electrolytes, liver and kidney function, and computed tomography or magnetic resonance imaging of the chest and abdomen. All patients had adrenal cancer confirmed by examination of the pathology specimens from their original surgery and all had clearly visible metastatic disease on CT and/or MRI. All patients except for two had been previously treated with at

least one other chemotherapeutic regimen; mitotane in 15 patients, suramin in one patient, and a combination of agents in three patients. Two patients refused treatment with available chemotherapeutic agents. All patients had normal electrolytes and all but one patient had normal renal function. Eleven patients with liver metastases had stable elevations of hepatic transaminases to two times normal levels.

Gossypol administration

Patients were given oral gossypol acetic acid (gossypol), 10 mg compressed tablets, beginning at a dose of 20 mg/d. This was increased by 10 mg/d every two to three days as tolerated up to a maintenance dose of 30-70 mg/d in divided doses. The first nine NIH patients were seen at least once a week during the loading phase, and every one to two weeks thereafter, to monitor side effects, routine blood chemistries, and gossypol levels. Five NIH patients were seen at least weekly during the loading phase and then were followed by their referring physicians, returning to the NIH every six weeks for follow up. The NIH patients recorded their side effects on symptom flow sheets where the presence and severity of ten different side effects were rated from 0-5 (0-not present, 1- mild, 2-moderate, 3-moderately severe, 4-severe,5-intolerable). In the NYH-CMC patients, side effects were monitored by patient interviews. Patients were seen at least once every two weeks to monitor side effects, routine blood chemistries, and gossypol levels. Patients who were on a stable gossypol dose were seen less often after the initial six week treatment period.

Gossypol levels and tumor response

Serum was collected every one to two weeks for measurement of gossypol levels during the loading phase and periodically after the first six weeks of treatment. 50 µl of 0.4M glutathione was added to 10 ml of heparinized blood and the serum was separated and frozen prior to analysis. The samples were analyzed by HPLC using a modification of the methods of Wu. et al (6). Specifically, samples were run over a Shodex column with a Waters 510 pump/712 WISP (Milford, MA) using a buffer containing 30% to 70% (v/v) ratio of 0.02M

phosphate and acetonitrile at a flow rate of 0.9 ml/min. The peaks of gossypol and gossypol dimethyl ether were determined using an ESA Coulochem Model 5100 detector (Bedford, MA) and the gossypol dimethyl ether was used as an internal control to determine the serum gossypol concentration. Serum gossypol levels were monitored in four patients following discontinuation of gossypol to estimate the half-time of disappearance of the drug.

CT or MRI scanning was performed every 4-6 weeks initially to monitor tumor response. Tumor volume was calculated from the dimensions of the lesions on CT or MRI scanning. Reduction in tumor volume by 50% or more was considered a partial response. Reduction in tumor volume by 10-50% was considered a minor response. Less than 10% change in tumor volume was considered stable disease and an increase in tumor volume was considered disease progression. Patients who had a tumor response or stabilization of disease were maintained on oral gossypol at a dose of 40-60 mg/d.

Results

Twenty-one patients with metastatic adrenal cancer received oral gossypol at doses of 30-70 mg/d (Table 1). The mean age of the patients was 39.2 +/- 18.9 years and the mean duration of illness was 2.8 +/- 2.2 years. The sites of disease were primarily lung and/or liver with or without abdominal recurrence. One patient had an isolated vertebral metastasis. Three patients received gossypol for less than four weeks due to the terminal nature of their illness (patients 1,2,and 8, Table 1). The gossypol levels in these patients were undetectable, and all three died of wide-spread disease within one month of stopping gossypol. These patients were judged to have had an inadequate trial of gossypol and were not included in the final analysis. Of the remaining eighteen patients, all completed at least six weeks of treatment and had detectable gossypol levels where measurements were available. Three of these eighteen patients, who had previously failed several other chemotherapeutic regimens, had partial tumor responses that will be described below. One patient had stable disease at the end of six weeks, but elected to stop gossypol due to difficulties in traveling to the NIH

for follow-up. One patient had a minor response and underwent surgical resection of her remaining lung metastases. Gossypol was discontinued in this patient as she had no visible disease to monitor following surgery. Thirteen patients had tumor progression and discontinued gossypol after 6-20 weeks.

Partial responses

The first response was seen in a 36 year old man with extensive pulmonary and hepatic metastases (Patient #4, Table 1) unresponsive to treatment with mitotane, suramin, and a combination of adriamycin and VP-16. His previous therapy had been complicated by polyneuropathy and cryptococcal sepsis. Prior to starting gossypol, he had shortness of breath requiring supplemental oxygen, decreased exercise tolerance, and abdominal pain. His physical exam revealed marked ascites and lower extremity edema. Four weeks after receiving gossypol at a dose of 50 mg/d, he had transient RUQ pain and transaminitis. CT scans at that time showed an 80-90% reduction in the volume of multiple hepatic lesions and greater than 90% reduction in the size of multiple pulmonary lesions (Figure 1). His tumor response was associated with decreased pain, ascites, and hepatomegaly. He experienced a marked improvement in exercise tolerance and no longer required supplemental oxygen. This clinical improvement lasted for eight months on oral gossypol at doses of 50-60 mg/d. During this time, however, his serum gossypol levels declined and his lesions began to regrow. Gossypol was discontinued after a total of nine months of treatment.

The second response was seen in a 54 year old woman who developed a bulky abdominal recurrence three years after her original adrenalectomy (Patient #5, Table 1). The tumor failed to respond to treatment with suramin and she presented with a large right-sided abdominal tumor mass and a small retroperitoneal metastasis. She complained of abdominal bloating and intermittent abdominal pain. Four weeks after beginning oral gossypol at a dose of 40 mg/d, she experienced sharp RUQ pain and right-sided pleuritic pain that resolved over a one week period. CT scans at that time showed no change in the retroperitoneal mass, but nearly complete central necrosis of the abdominal mass resulting in over 80%

reduction in the volume of the mass (Figure 2A). Following this tumor response, she had decreased abdominal pain and distention. Despite continued prescription of gossypol at doses of 40-50 mg/d, however, her serum gossypol concentration declined over the next four months. Although her abdominal lesion and clinical status remained stable, gossypol was discontinued after a total of five months due to growth of the retroperitoneal lesion.

The third response was seen in a 67 year old woman with a T12 paraspinal metastasis associated with severe lower back and leg pain (Patient #15, Table 1). The patient had previously failed treatment with mitotane. After 20 weeks of oral gossypol at a dose of 40 mg/d, there was a 50% reduction in the volume of the paraspinal lesion associated with a dramatic improvement in her back and leg pain (Figure 2B). The size of the lesion and the patients symptoms remain stable after one year on oral gossypol at a dose of 30 mg/d.

Gossypol levels and toxicity

The maximal serum gossypol levels achieved in the patients who received an adequate trial of gossypol were highly variable, ranging from 65 to 1,025 ng/dl (Table 1). The serum gossypol levels showed only a rough correlation with the prescribed gossypol dose and with the patients' side effects. In four patients who were followed after discontinuation of gossypol, the estimated half-time of disappearance was 2.9 +/- 0.9 weeks (Figure 3). The serum gossypol levels in patients who had tumor responses were 547, 465, and 83 ng/dl, respectively, at the time of their responses. The serum gossypol levels in patients who had tumor responses were indistinguishable from the gossypol levels in patients who did not respond.

The side effects of gossypol (and their incidence in the NIH patients) were xerostomia (93%), transient transaminitis (93%), dry skin (71%), fatigue (64%), intermittent nausea (36%), vomiting (21%), transient ileus (21%), and minor hair thinning (14%). In addition, one patient with pre-existing gynecomastia had increased size and tenderness of breast tissue while on gossypol therapy. Overall, the side effects of gossypol at doses of 0.6 to 0.8

mg/kg/d were well tolerated. Of the eighteen patients who had measurable gossypol levels, no patient had to permanently discontinue gossypol due to its side effects. Four patients had a transient episode of abdominal ileus after receiving gossypol continuously for three months at doses of 40 mg/d or more. The serum gossypol levels in these patients at the time they developed the ileus were 244, 351, 444, and 554 ng/dl. In all patients, the ileus resolved within one to two weeks when the drug was withheld and did not reoccur when the drug was restarted at a lower dose. Two of the non-responding patients developed hypokalemia (serum potassium = 2.3 and 2.6 mmol/L; normal range, 3.5 to 4.0 mmol/L) two to three weeks after discontinuing gossypol at a time when when they had severe hypercortisolism (urine free cortisol = 1850 and 11,800 nmol/d; normal range, 30-300 nmol/d). In both cases, the hypokalemia resolved with correction of the hypercortisolism.

Discussion

The prognosis for patients with adrenal cancer is dismal. The majority of patients present with advanced disease at the time of diagnosis. Surgical resection can prolong the survival in some patients, but most will eventually have inoperable disease and a five year survival less than 20%. In these patients, medical therapy has been generally disappointing. Mitotane can induce biochemical remissions in 60-70% of patients with hypercortisolism, but only 10-20% of patients have any objective decrease in tumor size (1, 9). The amount of tumor shrinkage with mitotane varies from 10-80% and these responses are generally short lived, lasting 6-9 months. The side effects of mitotane include fatigue, anorexia, nausea, vomiting, ataxia, dizziness, confusion, and memory loss. Various conventional chemotherapeutic agents have been used for the treatment of adrenal cancer including cisplatin, 5-fluorouracil, cytoxan, adriamycin, vincristine, and VP-16. The partial response rates for these regimens is 10-20% and the duration of response is one year on average (2, 10-13). The side effects associated with these chemotherapeutic regimens include myelosuppression, nausea, vomiting, alopecia, and cardiotoxicity. Thus, a drug with greater efficacy and less toxicity would be desirable for the treatment of metastatic adrenal cancer.

Gossypol is a naturally occurring biphenolic compound derived from crude cottonseed oil. It was first isolated as the cause of infertility in Chinese villages using crude cottonseed oil for cooking during times of economic deprivation. It has been shown to be a potent spermatotoxic agent as well as a general anti-metabolite (14-16). Gossypol has been shown to have *in vitro* activity against several tumor cell lines including mouse mammary carcinoma and human melanoma and colon carcinoma (17). Tso and colleagues examined the *in vivo* anti-tumor activity of gossypol in nude mice with Ehrlich ascites tumors (4). They found a significant increase in the survival of rats given daily intraperitoneal injections of gossypol at doses of 25, 50, and 100 μg/d compared to untreated animals. The therapeutic dose range was quite narrow, however, and the animals treated with 250 μg/d died from the toxic effects of gossypol. Rao and colleagues examined the effects of a single intraperitoneal injection of gossypol 48 hours after inoculation of mice with mouse mammary tumors (5). While 66% of animals were tumor free, 34% died of drug toxicity. By using oral gossypol, however, in nude mice bearing SW-13 human adrenal cancers, we were able to prevent death from drug toxicity while demonstrating inhibition of tumor growth and enhanced survival (3).

Based on these findings, we examined the effect of oral gossypol on adrenal cancer in humans. The largest studies using oral gossypol in humans are those done in China where it has been studied extensively as a potential male contraceptive agent (6, 7, 15). In these studies, over 8,00 normal men received oral gossypol at loading doses of 20 mg/d for several weeks followed by maintenance doses of 50-60 mg/week. The side effects were minimal and generally well tolerated. The only serious toxicity was profound hypokalemia in 0.1% of patients that was associated with reversible muscular paralysis in one patient. Since idiopathic hypokalemia also occurs in the normal population in China, the role of gossypol in hypokalemia is unclear. Hypokalemia was not noted in a subsequent series of patients receiving gossypol in South America (18).

Based on these contraceptive trials, we initiated our trial of gossypol in patients with adrenal cancer at a dose of 20 mg/d. There are no previous studies using larger doses of gossypol over an extended period of time. Thus, one of the objectives of this study was to

determine a safe dosage range. The maximum tolerated dose in our patients was 0.8 mg/kg/d. Doses above this were associated with excessive nausea, anorexia, and fatigue. In general, however, the side effects experienced by our patients were well tolerated. None of our patients experienced hypokalemia directly related to gossypol. Two patients had hypokalemia after discontinuation of gossypol, that was most likely due to excessive cortisol secretion. The potassium levels in these patients returned to normal after correction of their hypercortisolism. The only severe side effect of gossypol was a transient abdominal ileus that occurred in four patients treated for an extended period of time at doses over 40 mg/d. This responded to withholding the drug and reinstituting therapy at a lower dose.

The serum gossypol levels in our patients correlated only roughly with the prescribed dose and with the patients' side effects. The half-life of gossypol reported in animal studies is quite long, as was the half-time of drug disappearance in our patients. Thus, we suspect that, by increasing the dose every three days, a steady-state was not achieved and this was reflected by inconsistent drug levels. Also, gossypol is quite lipophilic, accumulating in the body fat, and thus serum gossypol levels may not reflect the body content of the drug. In addition, patient non-compliance may have been a factor in the discrepancy between the prescribed dose and serum drug levels. We were able to document one case where the prescribed dose was 40 mg/d and the patient was routinely taking 30 mg/d.

Partial tumor responses were seen at gossypol doses of 0.6-0.8 mg/kg/d. The serum gossypol levels at the time of these tumor responses, however, were quite variable, ranging from 83 to 547 ng/dl. Thus, we were unable to establish a minimum effective dosage of gossypol from our study. We were also unable to identify a minimum serum gossypol concentration that correlated with tumor response, because the serum gossypol concentrations in responders were indistinguishable from those in non-responders. In our experience higher doses of gossypol and higher gossypol concentrations did not correlate with increased tumor response. We therefore limited our study to doses of gossypol that could be tolerated in an outpatient setting and did not attempt to use higher doses in an inpatient setting with vigorous supportive measures such as parenteral feedings or intravenous anti-emetics. We

are unable to fully explain why, in two patients who initially had tumor shrinkage, the serum gossypol levels progressively declined despite continued prescription of gossypol at doses equal to or greater than those at the time of their response.

The mechanism of action of gossypol in adrenal cancer is unknown. Recently, Benz and colleagues studied the *in vitro* tumoricidal effects of gossypol on breast, ovarian, colon, and pancreatic cancer cell lines and demonstrated that the first ultrastructural change seen in these cells is the selective destruction of mitochondria, accompanied by a decrease in intracellular ATP. Thus, gossypol may exert its cytotoxic effects by uncoupling oxidative phosphorylation. It is unclear, however, how this activity could specifically target tumor cells *in vivo*. Another potential mechanism of gossypol action stems from its ability to inhibit endothelial-derived relaxing factor, an agent responsible for blood vessel dilatation. It is possible that, through this mechanism, gossypol interferes with tumor blood supply causing tumor necrosis. This could explain why one of our patients had nearly complete necrosis of a large abdominal lesion, but no effect on a small retroperitoneal lesion.

Overall, the partial response rate seen with oral gossypol was 17% and the duration of these responses was from several months to over one year. As with other chemotherapeutic trials in adrenal cancer, there was no placebo arm included in this study. Thus, we cannot determine the effect of gossypol on patient survival, or definitely rule out spontaneous tumor necrosis as a factor in the tumor responses. The response rate with gossypol treatment is similar to that seen with the other chemotherapeutic agents available for adrenal cancer, and confirms the generally poor response of adrenal cancer to medical therapies. The tumor responses seen during gossypol treatment occurred in patients who were refractory to other chemotherapeutic modalities. Thus, the response rate of gossypol needs to be viewed in the setting of a second line, salvage agent.

We conclude that gossypol can be used in relatively high oral doses for the treatment of adrenal cancer. The side effects are generally well tolerated, allowing it to be used daily on an outpatient basis. Although the partial response rate is low, it is comparable to the other

medical therapies available and it can be used as a salvage agent when other treatments for adrenal cancer have failed. This is the first demonstration of an *in vivo* anti tumor effect of gossypol in humans and indicates the need for further studies to elucidate the mechanism of gossypol action and explore its role in the treatment of other human cancers.

Figure legends.

Figure 1: Panel A: CT scans of the chest before (a) and during (b) treatment with gossypol (50 mg/d) in a 36 year old male with metastatic adrenal cancer. Panel B: CT scans of the liver before (a) and during (b) gossypol treatment in the same patient.

Figure 2: Panel A: CT scans of the abdomen before (a) and during (b) treatment with gossypol (40 mg/d) in a 54 year old woman with metastatic adrenal cancer. Panel B: MRI of the T12 vertebra before (a) and during (b) treatment with gossypol (40 mg/d) in a 67 year old woman with metastatic adrenal cancer.

Figure 3: Serum gossypol concentrations following discontinuation of gossypol in four patients with metastatic adrenal cancer. The mean half-time of disappearance (±SD) was 2.9 ± 0.9 weeks (range, 2.5-4.0 weeks).

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Table 1: Clinical characteristics and tumor response in 21 patients with metastatic adrenal cancer on oral gossypol.

Patient	Age	Sex	Duration of disease(years)	Sites of metastases	Prior Treatment	Gossypol dose(mg/d)	Duration of treatment (weeks)	Maximum. gossypol level(ng/ml)	Tumor response
1	55	M	4	Lung, liver	Mitotane	30	3*	<50	Progression
2	17	F	1.5	Lung, liver	Mitotane	40	3.5*	<50	Progression
3	28	M	1.5	Lung, liver, abd	Mitotane	70	6	1142	Progression
4	36	M	3.5	Lung, liver	Mitotane,Suramin	50	38	547	Partial response
5	62	F	4	Abd, liver	Adriamycin,VP-16 Suramin	40	20	465	Partial response
6	34	M	2	Lung, liver, abd	Mitotane,Suramin	50	16	344	Progression
7	34	M	1	Abd, liver	Mitotane	60	12	508	Progression
8	23	F	1	Lung, abd	Mitotane	40	3*	<50	Progression
9	23	F	1.5	Lung, liver	Mitotane	50	6	131	Progression
10	16	F	1	Liver	None	50	6	266	Progression
11	23	F	2.5	Abd, liver	Mitotane	50	6	N/A	Progression
12	30	F	2	Liver	Mitotane	50	12	242	Progression
13	22	F	3	Liver	Mitotane	50	12	175	Minor response
14	48	M	3	Lung, liver	Mitotane	50	6	95	Stablization
15	67	F	8	Vertebral bone	Mitotane	40	52	83	Partial response
16	82	F	1	Liver	Mitotane	40	6	552	Progression
17	73	F	2	Abd, liver	Mitotane	40	6	39#	Progression
18	90	M	4	Liver	Mitotane	40	6	449	Progression
19	51	F	9	Liver	Mitotane	40	6	65#	Progression
20	28	F	2	Liver, abd	Mitotane	40	6	N/A	Progression
21	52	M	1.5	Liver, abd	None	40	6	559	Progression

*Patients eliminated from the final analysis due to insufficient trial of gossypol.
Value obtained within the first three weeks of gossypol administration and does not reflect steady state levels

Acknowledgments

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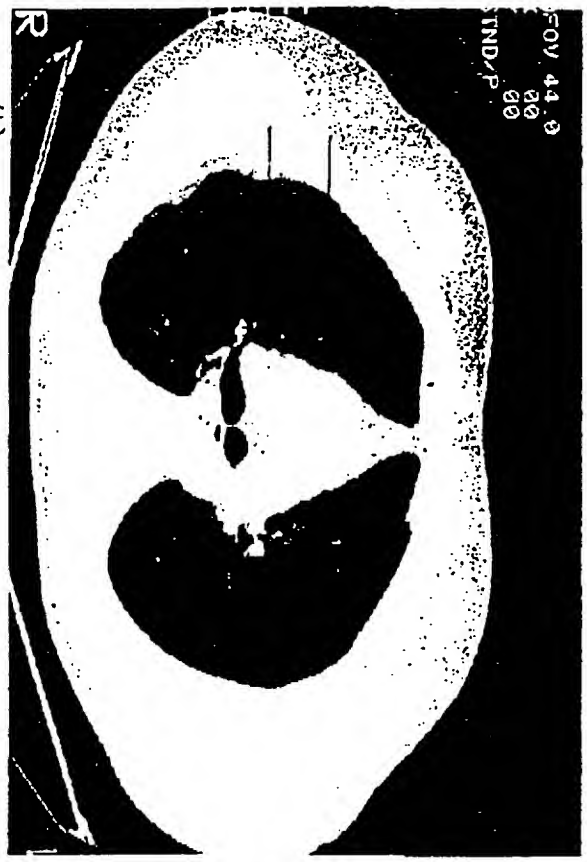
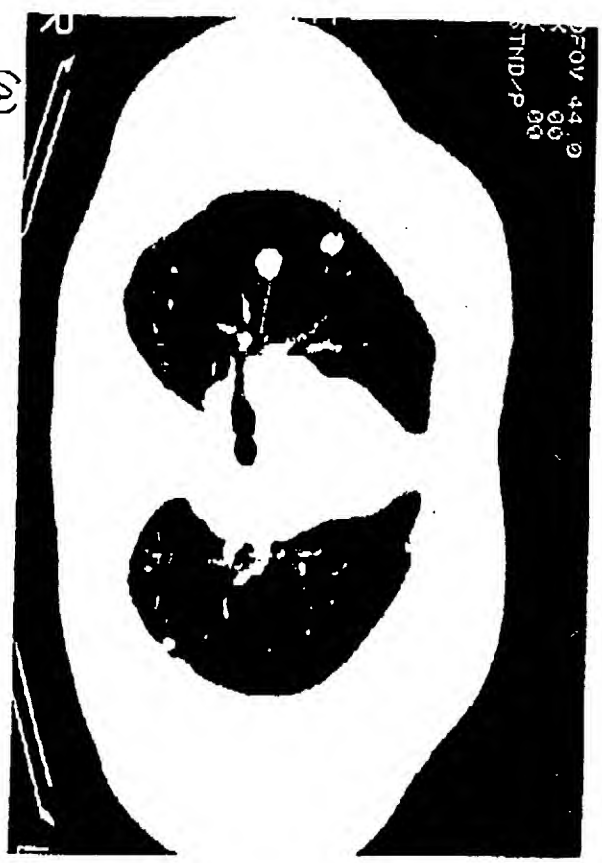
FIG 1

PANEL A



(b)



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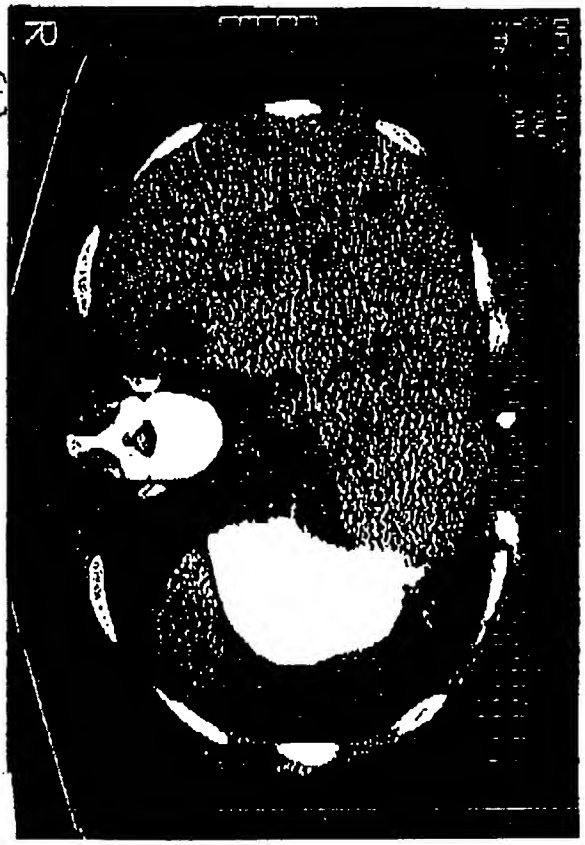
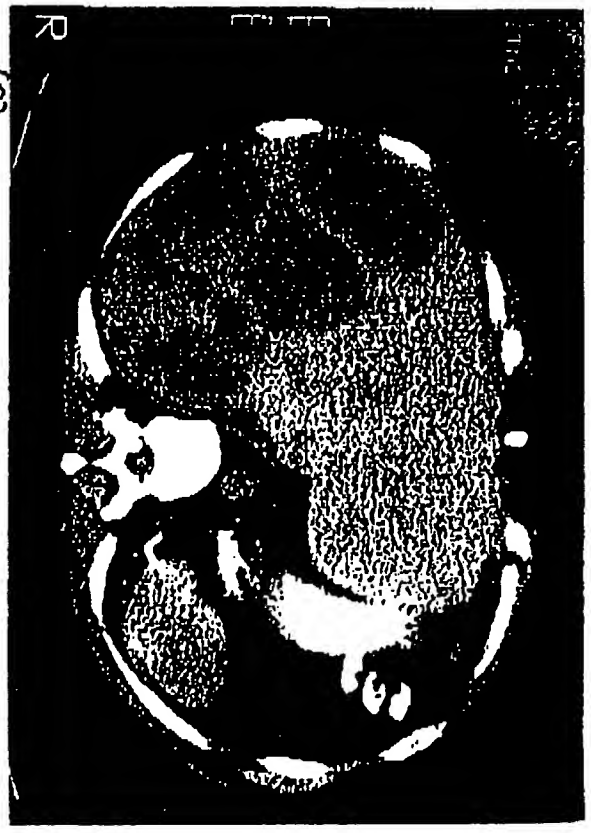


(b)

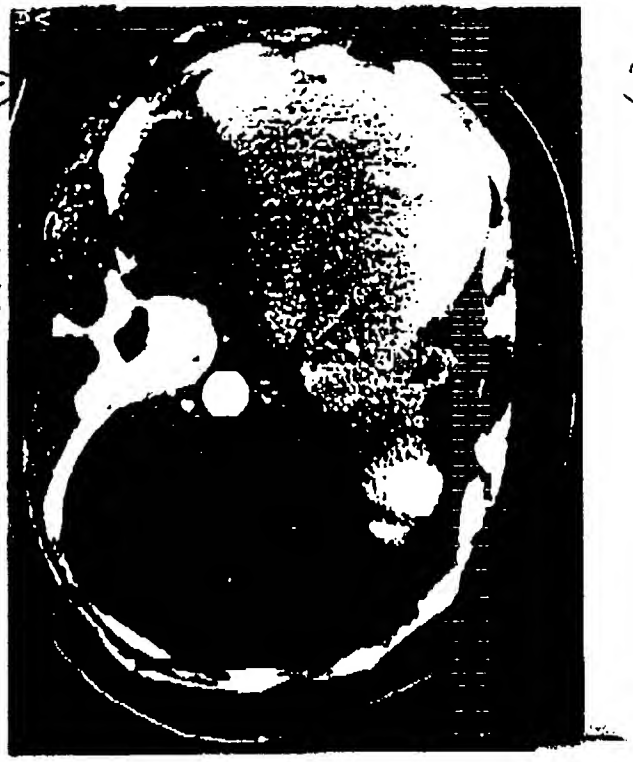


(a)

FIG 2

PANEL A

(b)



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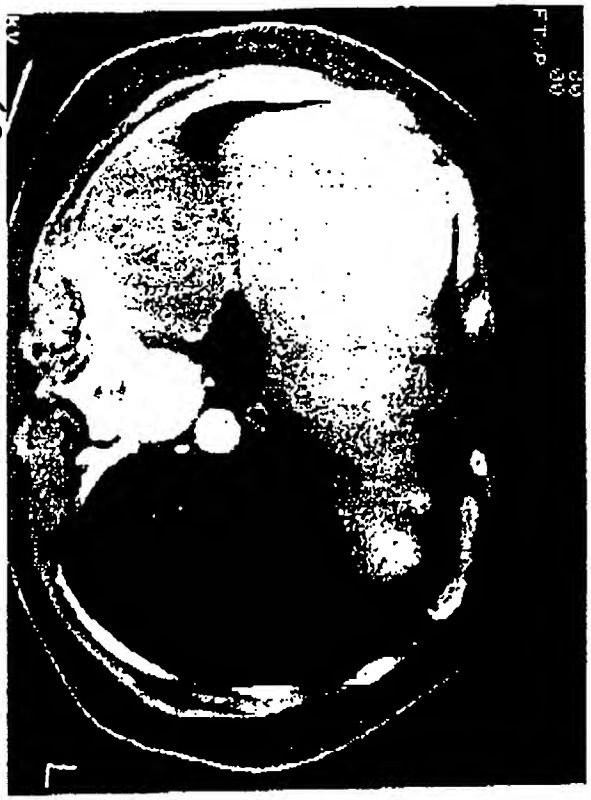

PANEL B

(b)

(a)

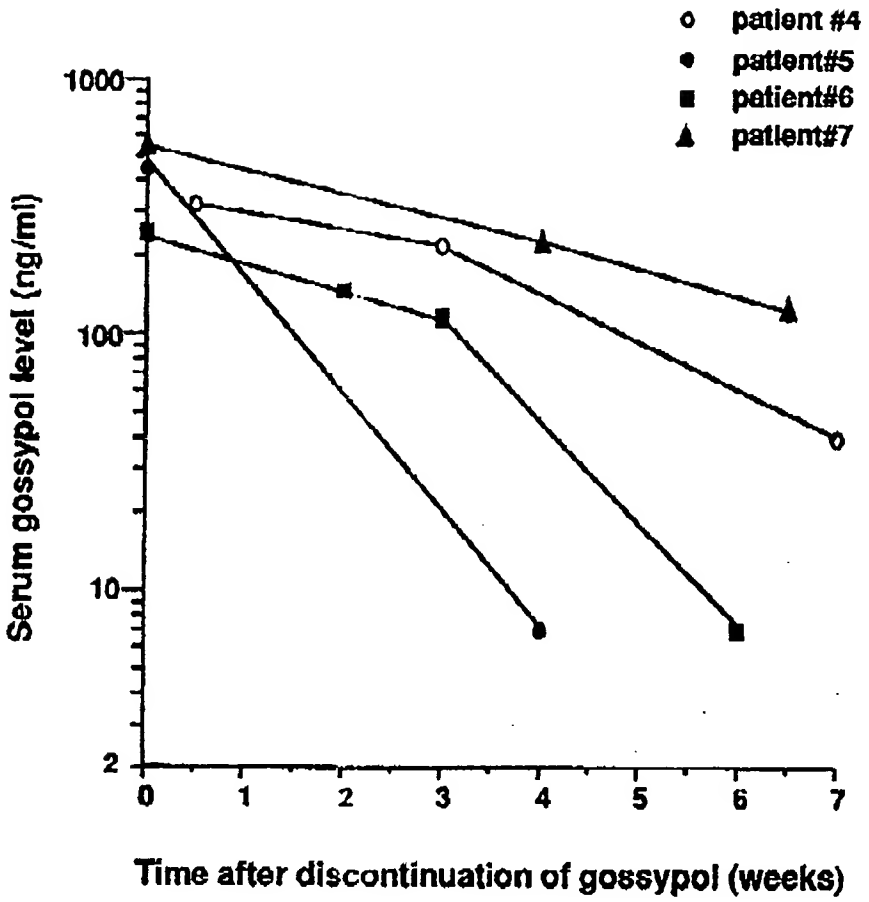
patient #4
patient#5
patient#6
patient#7
1000
100
10
2
Serum gossypol level (ng/ml)
0
1
2
3
4
5
6
7
Time after discontinuation of gossypol (weeks)

Fig 3

XXVI-3

Neoplasms of the Adrenal Cortex

Mary R. Flack
George P. Chrousos

Historical Perspective

Eustachius first described the "suprarenal" glands in 1563. It was not until the nineteenth century, however, that Cuvier isolated the adrenal cortex from the medulla (1805), and Arnold defined the various histologic zones of the adrenal cortex (1866).[60] The importance of the adrenal cortex for sustaining life was suggested by Addison in 1855 and confirmed the following year in animal studies by Brown-Sequard.[1,8] In 1927, Hartman showed that purified adrenal cortical extract could be used to treat adrenal insufficiency. The active substances in these adrenal extracts would later be identified as cortisol (1949) and aldosterone (1952).[60]

Harvey Cushing first described the clinical syndrome associated with excess adrenal secretion in 1910. He attributed the syndrome to basophilic adenomas of the pituitary gland, but in 1934 Walters described this same syndrome in patients with adrenal tumors. In 1811, Rolleston noted the association of adrenal tumors with virilization, and in 1890 Thompson demonstrated decreased virilization in a woman following resection of an adrenal tumor.[60] In 1952, Rapaport reported 188 cases of malignant adrenal tumors occurring between 1930–1949 associated with excess secretion of cortisol, androgens, and estrogens.[63]

Natural History and Staging

The natural history of adrenal cancer is dismal. The survival of untreated disease is generally less than three years.[11,56,60] Macfarlane reported a mean survival of only 2.9 months in 20 patients with surgically unresectable disease.[48] Most patients have either locally invasive disease or distant metastases at the time of diagnosis.[7,11,14,56,60,63] Even with surgical treatment the prognosis is generally poor. Although there are anecdotal reports of patients living 10–15 years with adrenocortical carcinoma,[63,275] the mean survival of patients following tumor resection is approximately 4 years.[11,31,32,45]

The staging system for adrenal cancer (Table XXVI-3-1) depends upon tumor size, nodal involvement, invasion of adjacent organs, and distant metastases.[48,58] Stage I disease refers to a tumor less than 5 cm in diameter that is confined to the adrenal gland. Stage I adrenal cancer is rare and can be difficult to distinguish from a benign adrenal adenoma.[29,63] When it occurs and there is complete resection, the prognosis is relatively good and long-term remissions have been reported.[5,24,48,58] Given the difficulty in distinguishing adenomas from carcinomas on pathologic criteria alone, however, some tumors classified as stage I carcinoma may actually be adrenal adenomas, which have an excellent prognosis following resection.

Table XXVI-3-1. Staging of Adrenocortical Carcinoma

Stage	T, N, M	Description
I	T1, N0, M0	Tumor less than 5 cm, confined to the adrenal gland
II	T2, N0, M0	Tumor greater than 5 cm, confined to the adrenal gland
III	T1 or T2, N1, M0	Tumor confined to the adrenal gland with involvement of local nodes or
	T3, N0, M0	Tumor extending beyond adrenal gland, but not invading adjacent organs
IV	T3 or T4, N1, M0 any T, M1	Tumor extending beyond adrenal, invading adjacent organs, with local node involvement or any tumor with metastases

Adpated from Macfarlane.[48]

Stage II disease refers to a tumor greater than 5 cm in diameter that is confined to the adrenal gland. Most patients with stage II disease will eventually have recurrent or metastatic disease, half of them within two years of tumor resection.[11] The likelihood of metastases is higher in patients with larger tumors, pathologic evidence of necrosis, vascular invasion, and increased mitotic activity. There is no single pathologic criterion, however, which accurately predicts recurrence in a given patient, except clear lymphatic or blood vessel invasion.[30,48,63] The overall five year survival for patients with stage II disease is 30–40% when all visible tumor is resected. There have been reports, however, of patients living 10–15 years after a complete resection.[1,11,15,24,56,60]

Seventy percent or more of the patients with adrenal cancer have either stage III or IV disease at the time of diagnosis.[7,11,14,56,60,63] Stage III disease refers to a tumor greater than 5 cm in diameter that is confined to the adrenal gland with involvement of adjacent nodes, or locally invasive disease without spread to adjacent organs. Despite complete resection, virtually 100% of patients with stage III disease

1

have recurrent and/or metastatic disease within 5 years of tumor resection.[7,11,36,53,58] Tumor necrosis, vascular invasion, nuclear mitoses, pleomorphism, and involvement of the *zona reticularis* have been reported to be poor prognostic signs.[30] The overall five year survival for stage III adrenal cancer is generally less than 30%.[11,45,56,60]

Stage IV disease refers to a tumor greater than 5 cm in diameter with invasion of adjacent organs and/or distant metastases. Adrenal cancer can spread directly to adjacent organs including the kidney, mesentery, posterior abdominal wall, pancreas, diaphragm, renal vein, and inferior vena cava.[26,31,42,48] Adrenal cancer can spread via the lymphatics to regional and para aortic lymph nodes, but more commonly spreads by the hematogenous route to distant organs. The most frequent sites of metastasis are lymph nodes (25–46%), lung (47–97%), liver (53–68%), abdomen (33–43%), and bone (11–33%). Metastases have been reported in the ovary, spleen, pleura, thyroid, pharyngeal tonsils, mediastinum, myocardium, brain, spinal cord, skin, and subcutaneous tissues.[11,31,42,48,56] The survival for patients with stage IV disease is extremely poor. The 5-year survival is generally less than 15% if all the tumor cannot be resected.[11,44,48,56,60] Luton reported a mean five-year survival of 22% in their patients with metastatic disease despite medical and surgical therapy; 25% for patients under age 40, and 15% for patients over age 40.[45]

Epidemiology

Adrenal carcinoma accounts for 0.05–0.2% of all cancers with a prevalence of two per million. A bimodal age distribution has been reported with one peak occurring before age 5, and the second in the fourth to fifth decade. Adrenal cancer, however, occurs at all ages from several months to the seventh and eighth decades.[31,42,48,53,56] In large cancer registries, there is a slight male predominance,[10,17,23] while in large clinical series there is a female predominance.[31,42,53] This is most likely due to the fact that secretory tumors, which are highly represented in clinical series, occur more commonly in women, while most adrenal cancers diagnosed in men are non-secretory.[48]

There are no known causative agents for adrenocortical carcinoma. There does not appear to be any association between chronic adrenal hyperplasia and the development of adrenal cancer. The majority of adrenal cancers occur sporadically. There have been a few reports, however, of adrenocortical cancer in patients with the Li-Fraumeni hereditary multiple tumor syndrome (breast cancer, soft tissue sarcomas, gliomas).[39,40,46,47]

Diagnosis

Despite the frequent association of adrenocortical carcinoma with endocrine hypersecretion, nearly half the patients with adrenal cancer have no recognizable endocrine syndrome.[11,48,56,62] These patients present with either abdominal pain or fullness or the incidental finding of an adrenal mass on radiologic studies done for other reasons.[26,38] Rarely, patients with adrenal cancer present with anorexia, weight loss, or fever of unknown origin, all of which are poor prognostic signs.[11,26,38] Although these patients have no identifiable endocrine syndrome, there may be elevation of urine or plasma steroids in 10–20% of these patients.[14,22,45,56,60]

Over 50% of patients with adrenal cancer have an associated endocrine syndrome such as Cushing's, virilization, Cushing's plus virilization, feminization, or hyperaldosteronism. These syndromes result from the secretion of cortisol and its precursors, adrenal androgens and their precursors, or rarely estrogen and aldosterone. Adrenal cancers are inefficient in their production of steroids. This results in the secretion of large amounts of steroid precursors relative to the amount of end product (Fig. XXVI-4-1).[9,25,41] Furthermore, the amount of steroids produced is often lower than expected for the large size of these tumors.

The most common syndrome associated with adrenal cancer is Cushing's syndrome caused by the excess secretion of cortisol and its precursors. It accounts for 30–40% of patients with a clinical syndrome.[31,42,53,63] Some of the typical signs and symptoms of Cushing's (Table XXVI-3-2) may be more subtle in patients with adrenal cancer, because of the inefficient steroidogenesis by many of these neoplasms. In women, hirsuitism and amenorrhea may be seen more frequently than with benign adrenal conditions due to the propensity for these tumors to secrete androgenic steroid precursors. Children rarely present with the classic clinical features of Cushing's syndrome; growth retardation and a weight gain that is inappropriate for their height are more common.[9,35,43]

Virilization is seen in 20–30% of those patients with an endocrine syndrome.[31,42,53,62] Virilization is rarely due to the secretion of testosterone itself, but is primarily associated with secretion of androgenic steroid precursors such as androstendione, dehydroxyepiandrosterone, and 17-hydroxyprogesterone.[31,42] The signs of symptoms of excess androgen secretion in females include increased libido, excessive muscle mass, temporal balding, cliteromegaly, and heterosexual precocious puberty in girls. In males, the clinical manifestations of excess androgen secretion are obscured, except for isosexual precocious puberty in boys (Table XXVI-3-2).

The combination of Cushing's syndrome and virilization occurs in 10–30% of those patients with an endocrine syndrome.[31,42,53,62] This combined syndrome is associated with the secretion of multiple steroids and their precursors including cortisol, androstenedione, 17-hydroxyprogesterone and dehydroepiandrosterone.[31,42] The diagnosis can only be made in women when cliteromegaly, temporal balding, or increased muscle mass are present, because amenorrhea and hirsuitism can be seen in Cushing's syndrome alone.

Feminization occurs in 5–10% of men with adrenal cancer.[31,42,53,62] This presents as decreased libido, impotence, gynecomastia, and testicular atrophy. Persistent questioning and careful examination may be required to elicit these findings, but they may be the only key to an early diagnosis in males who generally have more clinically silent tumors.[48]

Pure hyperaldosteronism is rare in adrenal cancer and accounts for five percent or less of those patients with an endocrine syndrome.[3,5,7,31,42] When it does occur, it presents with hypertension, hypokalemia, and a metabolic alkalosis. Hypertension and hypokalemia, however, can occur with

Table XXVI-3-2. Clinical Findings and Laboratory Studies Found in Patients With Secretory Adrenal Cancers

Endocrine Syndrome (%)	Clinical Findings	Laboratory Studies (S.I. Units)
Cushings (30%)	Weight gain (truncal, dorsocervical and supraclavicular). Moon facies, plethora, hypertension, striae, hirsutism, peripheral wasting and weakness, glucose intolerance, amenorrhea, acne, mental changes, osteoporosis, edema, hypokalemia[43]	Urine: 17-ketosteroids = 30–200 mg/d (> 100 μmol/d) 17-hydroxysteroids > 15 mg/g creat (> 30 μmol/d) free cortisol (UFC) > 200 μg/d (> 400 nmol/d) increased tetrahydro compound S Plasma: ACTH suppressed < 15 pg/ml (< 4 pmol/L) DHEA(S) > 3,500 ng/ml (> 10 mmol/L)
Virilization (20%)	Temporal balding, increased muscle mass, cliteromegaly, deepening of the voice in women, heterosexual precocious puberty in girls, isosexual precocious puberty in boys	Urine: 17-ketosteroids = 30–200 mg/24h (> 100 μmol/d) increased urinary pregnenalone Plasma (females): testosterone > 200 ng/dl (> 4 nmol/L) androstendione > 3.5 μg/L (> 12 nmol/L)
Cushing's and Virilization (30%)	Combination of the above; the finding of virilization or precocious puberty in the setting of Cushing's syndrome is highly suggestive of adrenal carcinoma	Combination of above
Feminization (15%)	Impotence, loss of libido, testicular atrophy, fatigue, inability to concentrate in men	Plasma: (males) Estrone > 100 pg/ml (> 300 pmol/L) Estradiol > 50 pg/ml (> 180 pmol/L)
Hyperaldosteronism (5%)	Hypertension, hypokalemia, metabolic alkalosis	Plasma (normal salt intake): aldosterone > 30 ng/dL (> 800 pmol/L)

other syndromes in adrenal cancer due to the excess secretion of mineralocorticoid precursors, such as 11-deoxycorticosterone and corticosterone. Even more unusual presentations of adrenal cancer which have been reported include hypoglycemia, insulin resistance, and polycythemia.[10,51]

Several laboratory studies are useful in confirming excessive steroid secretion in patients with suspected adrenal cancer. Hypercortisolism is most often confirmed by measuring the urine free cortisol (UFC) in an aliquot from a 24 hour urine collection. Over 90% of patients with Cushing's syndrome have UFC values greater than 200 mcg/24 hours, while 97% of normal individuals have UFC values less than 100 mcg/24 hours. Values between 100 and 200 mcg/24 hours can be seen in patients with obesity, depression, stress, or alcoholism.[13,43] In patients with ambiguous UFC results, an overnight dexamethasone suppression test may be helpful. This test involves the oral administration of 1 mg of dexamethasone at midnight and measurement of plasma cortisol at 8 a.m. Normal individuals have cortisol values less than 5 μg/dL following dexamethasone, while patients with Cushing's syndrome generally have values greater than 5 μg/dL.[13]

Additionally, an aliquot from a 24-hour urine collection should be sent for measurement of 17-hydroxysteroids, 17-ketosteroids and creatinine. Sixty percent of patients with adrenal cancer have elevated 17-hydroxysteroid excretion and over 70% of patients have elevated 17-ketosteroid excretion. Fifty percent of patients with adrenal cancer have increased levels of both 17-hydroxy and 17-ketosteroids.[14,24,31,42,51] Extreme elevations of urinary 17-ketosteroids are often seen in patients with adrenal cancer (up to 200 mg/dL). An unusually high level of urinary ketosteroids in a patient with hypercortisolism is more suggestive of malignant adrenal disease than a benign adrenal process.[9,21,35,41,42]

There are several other tests for the differential diagnosis of Cushing's syndrome once hypercortisolism has been established. A plasma ACTH level using a reliable radioimmunoassay (usually a two-site or "sandwich" assay) can distinguish patients with ACTH-dependent Cushing's syndrome (pituitary tumors or ectopic ACTH secretion) from those with ACTH independent Cushing's syndrome (adrenal tumors or micronodular adrenal disease). Patients with pituitary disease or ectopic ACTH secretion have normal or elevated ACTH levels, while patients with primary adrenal disease, including adrenal carcinoma have suppressed ACTH levels.[9,13,35,43] An undetectable ACTH level with the appropriate findings of a large irregular adrenal mass on computed tomography is virtually diagnostic of adrenocortical carcinoma.

The classic test for the differential diagnosis of Cushing's syndrome is the high dose dexamethasone suppression test. This test involves obtaining 24-hour urine collections for 6 consecutive days. Following 2 baseline days, dexamethasone is given orally; 0.5 mg every 6 hours for 48 hours, then 2.0 mg every 6 hours for 48 hours. Traditionally, a decline in 17-hydroxysteroid excretion to less than 50% of basal values indicates pituitary disease. Patients with adrenal cancer, however, do not have any significant decrease in their 17-hydroxysteroid excretion following high dose dexamethasone.[9,13,35,43] The high dose dexamethasone test and other tests recommended for the differential diagnosis of Cushing's syndrome, such as ovine corticotropin stimulation and inferior petrosal sinus sampling, are not essential in the diagnosis of adrenal cancer, however, if the imaging studies are diagnostic and the ACTH level is suppressed.

Several other plasma and urinary steroids are elevated in patients with adrenal cancer. They include dehydroxyepian-

drosterone (DHEA) and its sulfated derivative (DHEA-S), pregnenalone, and 17-hydroxypregnenalone in the plasma and the tetrahydro conjugate of 11-deoxycortisol (tetrahydrocompound S) in the urine (Figure XXVI-3-1).[41,42,49] While these are generally not essential in the work up of hypercortisolism, they may occasionally be a clue to the presence of adrenal malignancy in a patient with Cushing's syndrome.

The clinical diagnosis of virilization can be confirmed by measurement of plasma androstendione, testosterone, sex hormone binding globulin, and urinary 17-ketosteroids. Plasma levels of DHEA and DHEA-S are elevated in the majority of patients with adrenal cancer whether or not they have the clinical manifestations or virilization and/or Cushing's.[41,42,49,55] In contrast, patients with secretory adrenal adenomas have suppressed DHEA-S levels (see differential diagnosis).

The clinical diagnosis of feminization can be confirmed by measurement of elevated plasma estradiol and/or estrone. Hyperaldosteronism can be confirmed by measurement of elevated plasma aldosterone levels. Although usually not needed clinically, plasma levels of corticosterone and deoxycorticosterone are frequently elevated in patients with adrenal cancer and the clinical appearance of hyperaldosteronism.[3]

A specific diagnosis of adrenal cancer depends on the identification of an adrenal mass on computed tomography (CT) and/or magnetic resonance imaging (MRI). The finding on CT of a large unilateral adrenal mass, with irregular borders, is virtually diagnostic of adrenal cancer. If a smaller mass is present, it is more difficult to distinguish an adrenal cancer from an adrenal adenoma (see differential diagnosis). On MRI scanning, malignant adrenal lesions have an intermediate to high signal intensity on T2 weighted images, while non-functional adenomas have a low signal intensity and pheochromocytomas have an extremely hgh signal intensity (Figure XXV-4-2).[15,54] Iodo-cholesterol scanning shows poor adrenal uptake in adrenal cancer compared with the enhanced uptake which may be seen in adrenal hyperplasia. This study is rarely indicated, however, given the accuracy of CT and MRI.

Differential Diagnosis

"Incidentalomas"

There is a 0.5–1.0% incidence of unexpected adrenal lesions on CT or MRI scans of the upper abdomen after the age of forty. These lesions can represent benign adrenal adenomas, adrenal carcinomas, metastases from an unknown primary cancer, cysts, or rarely myelolipomas.[4,12,15] The incidental findings of these lesions has led to the diagnostic problem of distinguishing benign lesions from early adrenal cancer. With the increasing use of CT and MRI scanning, adrenal cancers as small as 3.5 cm have been identified. Resection of these small lesions will no doubt have a better prognosis, so the question of the malignant potential of these incidentally discovered lesions is important. Copeland has suggested that lesions greater than 6 cm should be considered to have high malignant potential.[12] Belldegrun and colleagues also found that most malignant lesions were greater than 6 cm in diameter, while nearly all lesions less than 6 cm were benign. However, they recommended that, due to the rare finding of small adrenal cancers, all lesions greater than three cm should be removed if there is no contraindication to surgery.[4]

The issue of how to proceed when an incidentaloma is discovered is controversial. The consensus of most studies[4,9,12] is that patients found to have an incidental adrenal lesion should have a preliminary screen for endocrine hypersecretion, including a complete history and physical examination and a 24-hour urine for free cortisol, 17-hydroxysteroids, 17-ketosteroids, and creatinine. If flushing or hypertension are present, urine metanephrines and catecholamines should also be included. If signs and symptoms of virilization, feminization, or hyperaldosteronism are present plasma levels of testosterone, androstenedione, DHEAS, or aldosterone should be obtained. If there is evidence of endocrine hypersecretion, the lesion should be resected.

If no endocrine hypersecretion is found, some would recommend an MRI of the adrenals.[15,54] In general, non-functioning adenomas have a low signal intensity on T2 weighted MRI; carcinomas and adrenal metastases have an intermediate to high signal intensity; while pheochromocytomas have an extremely high signal intensity. Identification of a pheochromocytoma on this study would be extremely important if manipulations such as fine needle biopsy were being considered. The absence of hypertension or the lack of elevated urine metanephrines does not rule out a pheochromocytoma in all cases. If the lesion is of intermediate to high signal intensity on T2 weighted MRI, it could still represent an adrenal metastasis from an unknown primary cancer. A reasonable effort should be made to exclude common adenocarcinomas (i.e., breast, lung, gastrointestinal tract).

If there is no endocrine syndrome or occult primary cancer found and the lesion is less than 3 cm, the patient can be reassured with perhaps one follow-up CT scan. If the lesion is 3–6 cm, there are several reasonable courses of action. If the CT appearance of the lesion is suggestive of a cyst, fine needle aspiration can be done with careful follow-up. If the patient is elderly, or there are contraindications to surgery, observation and careful follow-up are reasonable. In these cases, periodic assessment of the secretory status as well as the size of the mass should be performed, since

Figure XXVI-3-1. Biosynthetic pathway for the production of steroids in the adrenal cortex.

Figure XXVI-3-2. Adrenocortical carcinoma shown on A Computed tomography, B T1 weighted MRI (TR 300 ms TE 26 ms), showing signal intensity equal to liver tissue, C T2 weighted MRI (TR 1500 ms TI 100 ms), showing high signal intensity compared to liver, D T2 weighted MRI (TR 1510 ms TI 100 ms), showing the mass in relation to the upper pole of the kidney. (Courtesy of J. Doppman)

many tumors become secretory long before they lead to a clearly recognizable endocrine syndrome. If the patient is young and there are no contraindications, surgical resection should be undertaken for lesions greater than 6 cm or lesions greater than 3–4 cm which have suspicious features such as intermediate to high signal intensity on T2 weighted MRI, irregular borders or patchy contrast uptake on CT, or failure to take up iodocholesterol.[4,12,15,54]

Adrenal Adenoma

In general, adrenal adenomas are highly efficient steroid secretors and tend to produce a single end-product such as cortisol, testosterone, or aldosterone, rather than multiple steroid precursors as in adrenal carcinoma.[8,26,42] A small lesion on CT scan, which avidly takes up iodocholesterol, in the setting of high levels of steroid secretion is highly suggestive of an adrenal adenoma. Following resection of an adrenal tumor, it may still be difficult to distinguish benign from malignant lesions on pathologic criteria. Hough has suggested that the presence of a diffuse growth pattern, broad fibrous bands, tumor necrosis, frequent mitoses, or vascular invasion are highly correlated with malignancy.[30] Others have emphasized the correlation between the size and weight of the lesion and the potential for malignancy.[48,55,59,63] However, there is no single criterion which distinguishes a malignant from a benign lesion in a given patient other than local invasion or metastatic disease. Thus, lesions which have a number of suspicious features on pathologic examination should be followed every three months initially for evidence of recurrence.

Therapy

Surgical resection is the only therapy which has been demonstrated to prolong survival in adrenal cancer.[11,27,48,58] Stage I and II disease should be treated by complete resection and careful follow-up (every 3 months initially, followed by every 6–12 months). Stage III disease should be treated by resection of all visible tumor and careful follow-up. Early recognition of recurrent or metastatic disease is important since resection of isolated metastatic lesions has prolonged survival in some patients.[2] State IV disease should be treated by removal of as much tumor as possible, including resection of isolated metastases. Some centers recommend adjuvant medical treatment with mitotane following complete resection in stage III and IV disease to increase the duration between recurrences, although this has not been tested in a controlled study.[45,60]

Medical therapy is generally recommended when all the tumor cannot be removed. While partial responses have been reported with medical therapy, it is generally ineffective in prolonging overall survival in adrenal cancer. Ortho-para'DDD (Mitotane) at high oral doses (up to 15 grams/day) causes remission of hypercortisolism in 50–60% of patients with adrenal cancer and Cushing's syndrome.[32] Tumor responses, however, occur much less frequently. Initially, a 20–40% partial tumor response rate was reported with mitotane treatment and there were several anecdotal reports of complete tumor responses.[6,16,32,33] More recent studies, however, involving large numbers of patients and more objective criteria for response indicate a partial response rate less than 20%.[29,45] These responses are short-lived lasting 6–10 months. Unfortunately, mitotane treatment has no effect on survival and the high doses required have considerable toxicity, including nausea, vomiting, anorexia, dizziness, lethargy, fatigue, and blood dyscrasias.

Various chemotherapeutic regimens have been used for the treatment of metastatic adrenal cancer. These have included agents such as cisplatin, etoposide, 5-fluorouracil, doxorubicin, and melphalan. In general, the response rates are less than 20% and are short-lived.[25,28,34,57,61] Radiation therapy can be used in combination with chemotherapy for palliation, particularly with bone metastases.[62]

In addition to Mitotane, there are a number of other agents which can be used to treat hypercortisolism including metyrapone (250 mg QID), aminoglutethimide (250 mg QID), and ketoconazole (10 mg/kg/d). In some patients, particularly with Mitotane treatment, hydrocortisone (15 mg/square meter/d) or florinef (100–400 μg/d) may be required to prevent adrenal insufficiency. Mineralocorticoid antagonists, such as spironolactone, or androgen antagonists, such as flutamide may aid in controlling the signs and symptoms of mineralocorticoid or androgen excess.[35,43]

Perspectives

One of the major problems with chemotherapy for adrenal cancer is the development of drug resistance. A surface glycoprotein (p-glycoprotein or MDR-glycoprotein) has been identified which may shuttle chemotherapeutic agents out of tumors cells. This protein is expressed in high levels in normal adrenals and in adrenal cancers.[19,20] It may contribute to the development of resistance to drugs which are transported by this glycoprotein. Agents such as verapamil and amiodorone, used in combination with chemotherapy, competitively inhibit this glycoprotein and may prolong the action of the other chemotherapeutic agents.

Experimental therapies such as Suramin, a reverse transcriptase inhibitor, and Gossypol, a plant toxin are currently being tested. Suramin has caused remissions in four of six-

teen patients with adrenal cancer[37] and Gossypol has caused a partial remission in one patient with adrenal cancer.[16]

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